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Ribosomal Mechanics, Antibiotics, and GTP Hydrolysis

Minireview

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The ribosome is an extremely ancient, highly complex, molecular machine in which rRNAs and ribosomal proteins have coevolved over some 3 billion years in order to produce proteins efficiently and accurately. After four decades of dedicated, frequently demoralizing, but often very creative experimental work, the ribosome may finally be starting to reveal the secrets of its molecular mechanics. For those who have persevered in this endeavour, this year signals the beginning of an exciting period of enlightenment. Evidence for this is presented in the current issue of *Cell*, where Wimberly et al. (1999) have determined the crystal structure of a complex of ribosomal protein L11 and an rRNA fragment from the hyperthermophilic bacterium *Thermotoga maritima*, which is an essential part of an important functional center on the 50S subunit. It will be followed, shortly, by high-resolution structures of the ribosomal subunits.

It has long been known for bacterial ribosomes that the L11–rRNA complex is involved, in some way, in regulating several ribosomal factor–dependent processes, including (1) elongation factor Tu (EF-Tu)–dependent aminoacyl-tRNA binding; (2) elongation factor G (EF-G)–mediated translocation; (3) initiation factor 2–dependent fMet-tRNA binding; and (4) release factor–dependent termination, as well as (5) stringent factor-dependent synthesis of the "magic spots" ppGpp and ppGppp (Gale et al., 1981). Most of these factors are known to be G proteins, and their intrinsic GTPase activities are stimulated by a ribosomal site that includes the L11-rRNA. It is widely termed the "GTPase-associated site" and is assumed to undergo conformational changes that regulate ribosomal factor binding and GTP hydrolysis (Cundliffe, 1986). Although much progress has been made recently in characterizing functional conformational transitions that occur within the elongation factors EF-Tu and EF-G *on* the ribosome, little is known about those that occur *in* the ribosome (Nyborg and Liljas, 1998).

The "GTPase-associated site" has been characterized extensively by a variety of genetic, cross-linking, and chemical footprinting approaches, and it consists of two main ribosomal components. The first is a protein–rRNA cluster containing protein L11, an rRNA region extending from nucleotides 1030–1125 of *Escherichia coli* 23S rRNA, and a pentameric L10.$(L12)_4$ complex (Figure 1). The latter complex is composed of two L12 dimers that attach to the rRNA via L10, adjacent to L11. The L12 proteins dimerize through their N-terminal domains, producing a "stalk" structure on the 50S subunit with a flexible hinge region that probably allows the C-terminal domains of the dimers to move relative to one another and to the ribosomal surface (Traut et al., 1995). The rRNA region is also involved, together with L11, in the binding of two thiopeptide antibiotics, thiostrepton and micrococcin, which can impede ribosomal factor–dependent processes. The second component is the ribotoxin stem-loop (nucleotides 2645–2675 of *E. coli* 23S rRNA), where cytotoxic proteins, including α-sarcin and ricin, modify the rRNA loop and block ribosomal factor–dependent processes, at least for elongation factors that produce overlapping chemical footprints in this rRNA region (Munishkin and Wool, 1997; Wilson and Noller, 1998). This highly conserved site composed of two rRNA regions and their associated proteins regulates binding and GTPase activity of the ribosomal factors (Figure 1).

The Structure of the L11–rRNA Complex

The crystal structure of the L11–rRNA complex is a landmark costructure of a ribosomal protein and an rRNA fragment. Moreover, as an integral part of an important functional site on the 50S subunit, its structural and functional properties have been investigated in detail. Gratifyingly, the crystal structure reinforces much of the earlier binding and footprinting data and correlates closely with the genetic results. However, what was not predictable from the earlier studies is the large number of tertiary interactions present in the rRNA component and the high degree of complexity of the interactions between the C-terminal domain of L11 and the rRNA.

The secondary structure of the rRNA was derived earlier from compensatory base change analyses, and it contains four double helical elements located around a four-way junction. In the crystal structure, the coaxial stacking of the terminal stem with the A1095 stem-loop, and of the U1082 hairpin with the A1067 stem-loop, produces a two-domain rRNA structure with very tight interdomain packing (Figure 2A). This packing is maintained first by a minor groove "ribose zipper," involving multiple hydrogen bond interactions between 2′-hydroxyl groups of riboses and bases that link the terminal stem and the U1082 hairpin and, second, by tertiary interactions between the major grooves of the A1067 and A1095 stem-loops (Figure 2A). Several divalent metal ions are observed in the crystal structure, and, in particular, a cadmium ion (which may be replaced by another divalent ion in vivo) is located at the four-way junction and may correspond to one of two divalent cations that were previously shown to stabilize the tertiary structure of the rRNA (Bukhman and Draper, 1997).

Protein L11 exhibits two globular domains connected by a linker sequence. The structure of the C-terminal domain, which was solved earlier by NMR (Xing et al., 1997), makes extensive main-chain contacts with the rRNA backbone and 2′-hydroxyl groups of the distorted

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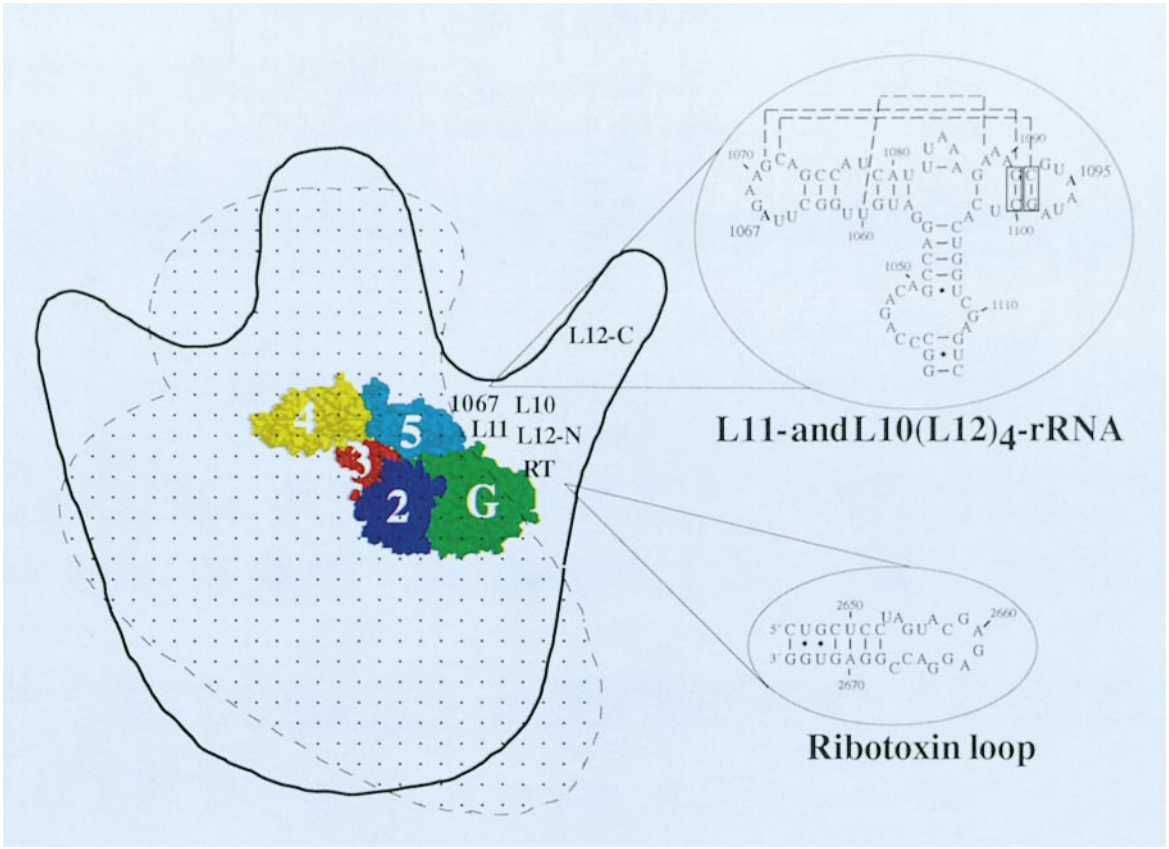

Figure 1. A Schematic Diagram of the *E. coli* Ribosome Complexed with EF-G

The 30S subunit (dotted) is superimposed on the 50S subunit with EF-G located in a cavity between the subunits. Whereas the G domain and domain 5 of EF-G face the 50S subunit and interact with the "GTPase-associated site" at the base of the L12 "stalk," domains 2 and 4 interact with the 30S subunit (Wilson and Noller, 1998). The main components of the "GTPase-associated site" are indicated on the 50S subunit surface. The insets show the secondary structures of the two rRNA components. One is the L11- and L10.$(L12)_4$-rRNA (1067), where L11 binds in the upper part and the binding of the pentamer is centered in the lower part of the rRNA. The other is the ribotoxin stem-loop (RT). L12-N and L12-C denote N- and C-terminal domains of L12, respectively.

minor groove of the A1067 stem-loop. This coincides closely with the L11-binding site that was deduced from chemical and hydroxyl radical footprinting results (Rosendahl and Douthwaite, 1993). The finding that this protein–rRNA interaction is mainly one of shape fitting, rather than side-chain base interactions, correlates with the finding that a fragment of yeast rRNA can replace the corresponding region within *E. coli* 23S rRNA (positions 1056–1103) in viable *E. coli* cells despite the 20 base differences between these rRNA regions (Thompson et al., 1993). In contrast, the N-terminal domain of L11 interacts exclusively, and weakly, with the A1067 and A1095 loop regions of the rRNA where thiopeptide antibiotics and EF-G are known to footprint (Figure 2A). Thus, the structure of the L11–rRNA complex provides important insight into how antibiotics bind and how they inhibit the functioning of ribosomal factors.

Antibiotic Binding

The antibiotics thiostrepton and micrococcin are complex thiopeptides that have partly similar structures and bind to the L11–rRNA complex. Both have recently been shown to be effective agents against the malaria parasite *Plasmodium falciparum* by inhibiting organelle protein biosynthesis. Resistance to these drugs is conferred by mutations at A1067 and/or A1095, in adjacent terminal loops, or by single-site mutations that occur on one face of the proline-rich helix in the N-terminal domain of L11 (Figure 2B) which is highly conserved in bacteria and archaea (Porse et al., 1998, 1999). 2′-*O* methylation of A1067 also provides natural resistance for the producer of thiostrepton *Streptomyces azureus*. Moreover, binding studies performed in vitro and in vivo show that thiostrepton and micrococcin generate strong chemical footprints in the A1067 and A1095 loops, where they produce similar effects at A1095 but opposite effects on the chemical reactivity of A1067.

It has been inferred, mainly on the bases of thiostrepton inhibition assays performed in vitro and mutational evidence, that the drugs inhibit protein synthesis during initiation, elongation, and/or termination (Gale et al., 1981). However, this inhibition has only been investigated rigorously for the EF-G-dependent reactions. Recently, it was proposed that while the drugs bind primarily to the rRNA, they cause inhibition by blocking a conformational change in the N-terminal domain of protein L11. The evidence for this derives from mutational studies and from an altered proteinase susceptibility observed at tyrosine-61 (tyrosine-62 in *E. coli* ribosomes)

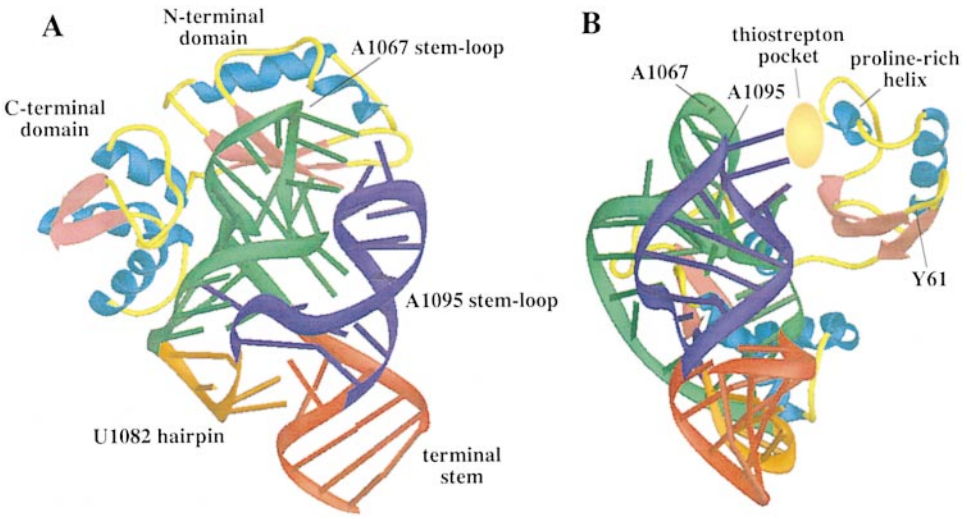

Figure 2. Two Views of the L11–rRNA Complex

(A and B) The crystal structure of the L11–rRNA complex containing nucleotides 1051–1108 of 23S rRNA (see Figure 1) (Wimberly et al., 1999). The four double helices of rRNA and the two globular domains of L11 are indicated. (B) emphasizes the putative antibiotic-binding cavity (denoted "thiostrepton pocket") that is flanked by A1067, A1095, and the proline-rich helix in the N-terminal domain of L11. We thank V. Ramakrishnan for providing an outline of the figure.

in the presence of thiostrepton (Porse et al., 1998, 1999). All of the mutations that confer antibiotic resistance are juxtaposed in the crystal structure in what looks like an antibiotic-binding pocket (labeled "thiostrepton pocket" in Figure 2B). Tantalizingly, the crystal structure of thiostrepton was determined some 30 years ago, but its orientation within the L11–rRNA complex is unknown. Nevertheless, it is clear from the structure that binding of the antibiotic in this pocket could lock the protein–rRNA structure and prevent a functional conformational change in the L11 protein and/or rRNA. Wimberly et al. (1999) note a potential mechanism whereby the thiazole groups of the two drugs could mimic the multiple proline residues of L11 and compete for a common ribosomal- or factor-binding site.

Importantly, blockage of such functional conformational switches may be a common mechanism for inhibition of protein biosynthesis by other antibiotics. For example, both fusidic acid and kirromycin, which stall EF-G and EF-Tu, respectively, on the ribosome after GTP hydrolysis, are considered to inhibit the interdomain rearrangements of the factors that are a prerequisite for their release from the ribosome.

How Do the Elongation Factors Interact with the Ribosomal Site?

Crystal structures of the elongation factors reveal that the overall shapes of the ternary complex of aminoacyl-tRNA.EF-Tu.GTP and EF-G.GDP are very similar (Nyborg and Liljas, 1998). The EF-G exhibits five main structural domains (Figure 1), where domain 4 corresponds to the anticodon stem-loop of the tRNA in the ternary complex. In line with this structural mimicry, cryo-electron microscopy studies show that the elongation factors occupy almost identical positions on the ribosome (reviewed in Nyborg and Liljas, 1998). Both elongation factors contact the "GTPase-associated site" at the base of the L12 "stalk" on the large subunit via their G domains, which bind GTP and GDP. Moreover, domain 5 of EF-G, which has no counterpart in EF-Tu, makes additional contacts in the same region. This interpretation is reinforced by the results of chemical footprinting and hydroxyl radical probing, which suggest that the G domains of the factors interact with the ribotoxin loop, while domain 5 of EF-G binds to the A1067 stem-loop (Wilson and Noller, 1998). Thus, although the overall architecture of the complexes are similar, contacts with the "GTPase-associated site" differ, and this may have important functional implications.

Evidence for a Ribosomal Switch

There is strong evidence for a reciprocating transition occurring in the ribosome that affects binding and release of the elongation factors. It derives from the demonstration that, on a tRNA-free *E. coli* ribosome, a synergistic effect occurs between the GTPase activities of EF-Tu and EF-G (Mesters et al., 1994). This implies that a ribosomal site oscillates between two conformers, triggered by GTP hydrolysis, which facilitates the exchange of elongation factors. The finding that this effect was inhibited by thiostrepton directly implicates the L11–rRNA region in some reciprocal movement on the ribosome between an EF-Tu-GTP binding conformer and an EF-G-GTP binding conformer.

The structure of the L11–rRNA complex suggests a basis for such a molecular switch mechanism. While the C-terminal domain of L11 is anchored firmly on the distorted minor groove of the A1067 stem-loop, the N-terminal domain interacts weakly with the A1067 and A1095 loops, suggesting that the latter may alternate between rRNA-bound and rRNA-free states or, alternatively, assume altered conformations on the rRNA (Figure 2B). Such a switch could regulate binding and release of the ribosomal factors (Cundliffe, 1986).

The following presents a model for how this molecular switch could regulate elongation factor binding to the

ribosome. Chemical footprinting and cross-linking data suggest that EF-G, in contrast to EF-Tu, interacts specifically with the A1067 loop such that the N-terminal domain of L11 may regulate its binding. Thus, EF-G binding would only occur in the rRNA-free state of the N-terminal domain of L11. GTP hydrolysis, which is likely to be directly stimulated via an interaction between the ribotoxin stem-loop and the G domain of EF-G, possibly facilitated by the L10.$(L12)_4$ pentamer, would then produce a switch to the RNA-bound state of the N-terminal domain of L11, causing domain 5 of EF-G to dissociate from the L11–rRNA site. This, in turn, would trigger movement of EF-G, and of the ribosome-bound tRNA–mRNA complex, in the translocation step. Since there is no direct evidence for an interaction between EF-Tu and the L11-rRNA, although thiostrepton inhibits mRNA-directed ternary complex binding (Hornig et al., 1987), binding of the ternary complex may occur independently of these putative reciprocal conformers. However, EF-Tu-dependent GTP hydrolysis, and release of EF-Tu-GDP, must leave the N-terminal domain of L11 in the RNA-free state to prepare the ribosome for binding of EF-G-GTP. Release of EF-Tu-GDP, after delivery of the aminoacyl-tRNA substrate to the ribosome, in contrast to that of EF-G-GDP, is likely to be facilitated by the large structural rearrangement within the factor on GTP hydrolysis (Nyborg and Liljas, 1998).

The pentameric L10.$(L12)_4$ complex is likely to be involved in mediating such a conformational switch in the L11–rRNA complex. It interacts with the ribosomal factors, and it binds cooperatively with, and adjacent to, L11 on the rRNA (Figure 1). This suggests either that L11 and L10.$(L12)_4$ interact directly or that allosteric effects occur through the rRNA. In any event, the design and conservation of the pentameric L10.$(L12)_4$ complex make it a likely contributor to movement (Traut et al., 1995).

Afterthoughts

As usual, interpreting ribosomal function is never straightforward. Thus, although characterization of viable L11-minus mutants in bacteria and archaea undermines, to a degree, the preceding discussion, the highly retarded growth phenotypes of such mutants demonstrate that the proposed role of protein L11 in the ribosomal switch mechanism is important. However, it is not absolutely essential for protein biosynthesis, which lends support to the view that a primitive ribosome was essentially an RNA machine. A further complication is the situation in eukaryotic ribosomes, where the rRNA secondary structure, the L11 sequence, and the elongation factor sequences show high conservation, especially with their archaeal homologs, but crucially the eukaryotic L11 lacks the proline-rich helix that contributes to the antibiotic pocket. While this explains the lack of sensitivity of eukaryotic ribosomes to thiostrepton and micrococcin, it also suggests that the mechanism of a conformational switch may differ in some significant way in eukaryotes.

The challenge for the future—the most difficult of problems for the molecular biologist—is to unravel the order and interdependence of the movements outlined above. In particular, this involves elucidating the details of the molecular mechanical interplay between the ribosomal "GTP-associated site" and the ribosome-bound tRNAs and factors. The eagerly awaited crystal structures of the ribosomal subunits will at least provide a basis for designing meaningful experiments.

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